Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Absence of REV3L promotes p53-regulated cancer cell metabolism in cisplatin-treated lung carcinoma cells

Linghao Kong [a, b, 1], Michael M. Murata [a, 1], Michelle A. Digman [a, *]

[a] Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, CA 92697, USA
[b] University High School, Irvine, CA 92612, USA

ARTICLE INFO

Article history:
Received 22 December 2017
Accepted 3 January 2018
Available online xxx

Keywords:
Reversionless 3-like protein (REV3L)
p53
Reduced nicotinamide adenine dinucleotide (NADH)
Fluorescence lifetime imaging microscopy (FLIM)
Oxidative phosphorylation (oxphos)
Cisplatin

ABSTRACT

Lung cancer is one of the deadliest cancers in the world because of chemo-resistance to the commonly used cisplatin-based treatments. The use of low fidelity DNA polymerases in the translesional synthesis (TLS) DNA damage response pathway that repairs lesions caused by cisplatin also presents a mutational carcinogenic burden on cells that needs to be regulated by the tumor suppressor protein p53. However, there is much debate over the roles of the reversionless 3-like (REV3L) protein responsible for TLS and p53 in regulating cancer cell metabolism. In this study, the fluorescence lifetime of the metabolic co-enzyme NADH reveals that the absence of REV3L can promote the p53-mediated upregulation of oxidative phosphorylation in cisplatin-treated H1299 lung carcinoma cells and increases cancer cell sensitivity to this platinum-based chemotherapy. These results demonstrate a previously unrecognized relationship between p53 and REV3L in cancer cell metabolism and may lead to improvements in chemotherapy treatment plans that reduce cisplatin resistance in lung cancer.

1. Introduction

Lung carcinoma is one of the deadliest cancers in the world [1]. Cisplatin-based combination chemotherapy is one of the most common methods for managing and treating lung cancer. Cisplatin reduces cancer cell proliferation by creating DNA crosslinks that stall replication forks, which are highly toxic to rapidly dividing cells. The covalent bonds formed at these crosslinks inhibit motion of DNA polymerases at replication forks, forcing the polymerases to be ejected from the DNA strand [2]. These DNA adducts initiate the DNA damage response (DDR), activating the tumor suppressor protein p53. Activation of p53 leads to processes that regulate metabolism, limit proliferation, and resist carcinogenesis [3]. Accumulation of cisplatin adducts repeatedly activates DNA damage signaling, which reduces cyclin-dependent kinase activity leading to cell cycle arrest [4].

Patients typically have favorable initial responses to cisplatin-based regimens, but these are brief and offer only marginal improvement due to the development of chemotherapy-resistance. Several mechanisms of resistance have been proposed, including changes in DNA methylation, alterations of membrane protein trafficking, and increased DNA repair [5]. In particular, the activation of DNA damage responses at DNA lesions such as cisplatin-induced crosslinks prevent replication fork collapse and promote survival, eventually leading to cisplatin resistance [6]. These bulky DNA lesions are processed by translesional DNA synthesis (TLS) and mediated by specialized DNA polymerases, such as Pol ζ. Unlike the highly accurate DNA polymerases used in replication, such as Pol δ, Pol ζ has low fidelity nucleotide insertion. The reversionless 3-like (REV3L) protein is the catalytic subunit of Pol ζ that is responsible for TLS [7]. REV3L has been shown to increase cancer cell viability, especially following cisplatin-induced damage [8–10].

The use of low fidelity DNA polymerases in TLS presents a mutational carcinogenic burden on cells that needs to be regulated by the tumor suppressor protein p53. In addition to suppressing TLS, p53 has many key functions in resisting carcinogenesis including cell cycle arrest, apoptosis, and metabolic regulation. Without p53, cancer cells can proceed with high rates of unregulated glycolysis, known as the Warburg effect and is one of the hallmarks of cancer [11]. This p53-mediated regulation of

Abbreviations: REV3L, reversionless-3 like; TLS, translesional DNA synthesis; FLIM, fluorescence lifetime imaging and microscopy; NADH, reduced nicotinamide adenine dinucleotide; Oxphos, oxidative phosphorylation; R + AA, rotenone and antimycin A; DG + DCA, 2-deoxy-D-gluocose and dichloroacetate; DDR, DNA damage response.

* Corresponding author.
E-mail address: mdigman@uci.edu (M.A. Digman).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.01.026
0006-291X/

metabolism occurs through its promotion of target genes such as glutaminase 2 and TIGAR to upregulate oxphos and down-regulate glycolysis, respectively [12]. Furthermore, REV3L may also be involved in metabolic regulation, but its effects on metabolism are still debated with some studies suggesting that REV3L increases reliance on glycolysis and others demonstrating that it promotes oxphos [9,13]. Because the one of the hallmarks of cancer cells is Warburg metabolism, understanding the mechanisms that limit the cancerous metabolic phenotype may lead to improvements in chemotherapy treatment plans that reduce cisplatin resistance in cancer cells. Indeed, the expression of p53 in p53-null cisplatin-resistant cell lines has been shown to increase sensitivity to cisplatin [14]. In this study, the absence of REV3L can promote the p53-mediated upregulation of oxphos in cisplatin-treated H1299 lung carcinoma cells and increases cancer cell sensitivity to this platinum-based chemotherapy.

2. Materials and methods

2.1. Cell culture, plasmids, transfections, and treatments

H1299 cells (ATCC) were cultured in RPMI (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were trypsinized and plated at 60–80% confluency on a glass-bottomed imaging dish. The EGFP and p53-GFP plasmids are commercially available through Addgene while the p53-R175H-GFP plasmid was kindly constructed and provided by Dr. Lee Bardwell and Dr. Jane Bardwell in the Department of Developmental and Cell Biology at the University of California, Irvine. Lipofectamine 3000 (Invitrogen, Carlsbad, CA) was used to transfect according to manufacturer's instructions. Cells were incubated at 37 °C and 5% CO_2 for 12–24 h prior to imaging. To induce intra-strand crosslinks, cells were treated with 20 μM cis-diamminedichloroplatinum (II) (cisplatin) (Sigma-Aldrich, St. Louis, MO) for the duration of the experiments. For inhibition of oxidative phosphorylation, cells were treated with 10 μM rotenone and 10 μM antimycin A (Sigma-Aldrich, St. Louis, MO) in 0.35% DMSO for 1 h prior to imaging. For inhibition of glycolysis, cells were treated with 20 mM 2-deoxy-D-glucose and 5 mM dichloroacetate (Sigma-Aldrich, St. Louis, MO) for 6 h prior to imaging.

2.2. shRNA, siRNA, and western blot

Short hairpin RNA (shRNA) against REV3L (shREV3L) (pSuperior.puro-shREV3-4, Addgene #38029) and short interfering RNA (siRNA) against REV3L (siREV3L) (UrCUrGrGrCrGrCUrGUrCrArArGUUCTT) were used to silence the *REV3L* gene. The shRNA control (shControl) is commercially available at Sigma and the siRNA control (siControl) is commercially available at QIAGEN. Transfections of shRNA and siRNA were done following DNA plasmid transfection but had identical steps, with shRNA or siRNA in lieu of the DNA plasmid. 1 μg of shRNA or 1 μL of 20 μM siRNA was used for the transfection of each imaging dish. 24 h after shRNA transfection, 2.0 μg/mL puromycin RPMI 1640 media was applied for 24 h to select for successfully transfected shRNA cells. Cells were harvested for Western blot analysis or imaged approximately 24 h after the final transfection. Whole cell lysates prepared with RIPA buffer were subjected to SDS-PAGE followed by Western blot analysis with the anti-REV3L antibody (MyBioSource) and the anti-β tubulin antibody (Sigma-Aldrich, St. Louis, MO) for the loading control.

2.3. Instrumentation and data analysis

Confocal and fluorescence lifetime imaging microscopy (FLIM) experiments were performed on an inverted confocal Zeiss LSM710 (Carl Zeiss, Jena, Germany) with a 40 × 1.2NA water-immersion objective (Zeiss, Korr C-Apochromat). Green fluorescent protein (GFP) excitation was achieved using a one-photon argon ion laser at 488 nm and emission was captured at 500–600 nm. In FLIM experiments, a Mai Tai titanium-sapphire 100 femto-second pulsed laser at 80 MHz (Spectra-Physics, Santa Clara, CA) was used for sample excitation. An ISS A320 FastFLIM box (ISS, Champaign, IL) and a photomultiplier tube (H7422P-40, Hamamatsu Photonics, Hamamatsu, Japan) were used for data acquisition. FLIM images were acquired at 740 nm two-photon excitation with image sizes of 256 × 256 pixels and a scan speed of 25.21 μs/pixel. Fluorescence signal was captured at 420–500 nm for NADH auto-fluorescence. Instrument response time was referenced using coumarin-6 in pure ethanol, which has a known single exponential lifetime of 2.5 ns FLIM data was processed in the SimFCS software developed at the Laboratory for Fluorescence Dynamics, University of California, Irvine as previously described [15].

2.4. Cell viability assay

Cells were plated onto gridded imaging dishes to determine cell survival following cisplatin treatment using morphology. Cell viability was measured by vital dye exclusion by propidium iodide (0.8 μg/mL) and total cell count was determined by Hoechst 33342 (0.5 μg/mL).

3. Results

3.1. p53 upregulates oxidative phosphorylation in H1299 cells

The tumor suppressor p53 has been known to regulate metabolism through the upregulation of oxphos and the down-regulation of glycolysis. In some situations, however, it has also been known to upregulate glycolysis [3]. We first sought to elucidate the impact of p53 on the fraction of protein-bound NADH in H1299 cancer cells, which can be indicative of the overall metabolic state of the cell. The p53-null H1299 lung carcinoma cells were transfected with wild type p53 (p53-GFP) or the EGFP control. Fluorescence lifetime data of NADH in H1299 cells was acquired to observe changes in the fraction of bound NADH. Previous studies have demonstrated that the phasor approach to fluorescence lifetime analysis provides a graphical representation of lifetime data and by using 740 nm excitation with a bandpass filter, the fluorescence signal from NADH can be isolated. Here, FLIM data of NADH was collected and transformed to coordinates on the phasor plot as previously described (Fig. 1A) [15]. Once the phasor positions of freely floating NADH and protein-bound NADH are established, the fraction of bound NADH can be determined by the linear combination of the phasors, which follow the rules of vector addition [16]. Images were pseudo-colored based on the fluorescence lifetime along this linear combinatorial trajectory with shorter lifetimes colored red and longer lifetimes colored white to illustrate free and bound NADH, respectively. Brightfield images for both EGFP and p53-GFP cells were taken to demonstrate the nuclear localization of p53 (Fig. 1B). Pseudo-colored FLIM images of H1299 cells shows the sub-cellular distribution of NADH and that p53 induces a shift toward bound NADH in the cytoplasmic and nuclear compartments. H1299 cells expressing p53 exhibited a significantly higher fraction of bound NADH relative to control p53-null cells. This difference was observed in both the nuclear and the cytoplasmic sub-cellular compartments (Fig. 1C).

To determine whether or not the fraction of bound NADH reflects the cellular metabolic state of H1299 cells, combinations of mitochondrial and glycolytic inhibitors were used as demonstrated previously [17]. For inhibition of mitochondrial oxidative

Please cite this article in press as: L. Kong, et al., Absence of REV3L promotes p53-regulated cancer cell metabolism in cisplatin-treated lung carcinoma cells, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2018.01.026